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Identification of chemical compounds from agarwood hydrosol (*Aquilaria malaccensis*) fruits via LC-QTOF-MS/MS analysis

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Abstract. Gaharu hydrosol is being considered as a by-product produced during the hydrodistillation of resinous wood part of Aquilaria spp. Agarwood hydrosol was reported to possess many bioactive compounds that are beneficial for health. However, current studies on the chemical composition of agarwood hydrosol from the fruit part are still lacking. This research presents the untargeted chemical compound of agarwood hydrosol from *Aquilaria malaccensis* fruit (AF) via liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS) and comparison the active functional groups with industrial hydrosol grade using Fourier transform infrared (FTIR). Qualitative tandem LC-QTOF-MS/MS was utilised to identify compounds in the extracted sample. The data processing revealed the presence of 128 known compounds in the hydrosol from A. malaccensis fruit in negative ionization mode and only one chemical profile detected after switched to positive ionization mode. This result contains the retention times value of m/z [M - H-], [M + HCOO-], [M + H+] and similar database search hit identities of the 129 compounds detected during the LC-QTOFMS/MS analysis in Table A1 and A2.

1. Introduction

Nowadays, Agarwood is one of the typical plants that are highly in demand in the world [1]. Agarwood is a resin-impregnated heartwood of Aquilaria species from Thymelaeaceae family that encompasses about 15 species in tropical Asia [2]. *Aquilaria malaccensis* is one of the species that was found and grows in Malaysia. Agarwood and its essential oil have been used and known a long time ago to have medicinal properties and used in traditional medicine, pharmaceutical; incense in religious practice and mostly in perfume. Hashim et al. (2019) discussed on agarwood used as medicine in traditional practices as well as their pharmacologic pieces of evidence in modern science [3]. Several researchers reported that this plant contained more than 60 chemical compounds. Therefore, the presence of the bioactive compounds in Agarwood trees such as mangiferin, genkwanin 5-O-β-primeveroside and iriflophenone 3,5-C-β-diglucoside can be used as herbal supplement [4]. Among all the compounds, mangiferin has a wide range of pharmacological effects such as anti-HIV, antioxidant, antidiabetic and anticancer activity [5].

The standard extraction method for the extraction of essential oil from agarwood wood parts was hydrodistillation. In this process, by-product is produced known as hydrosol (distillate fraction), that is used in traditional medicine and aromatherapy. Hydrosol is made up of water-soluble non-volatile organic compounds and may contain a small amount of essential oil. Agarwood hydrosol is sold as health supplement in Malaysia, claimed to be used in cancer treatment [6]. Unfortunately, the literature on chemical profile in agarwood hydrosols and the use of hydrosol in human health from the fruit parts are scarce.

Thus, this study aimed to provide information about the chemical profiles in agarwood hydrosol from the fruit parts obtained by liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS). This data can help researchers in herbal medicinal plant to design effective drug discovery assays for the discovery of new therapeutic applications of the compounds.

2. Materials and method

All the materials and method used in this study will be explained further in this section.

2.1 Materials and equipments used

The fresh fruits of agarwood species of *A. malaccensis* were obtained from Pahang. The fruits' species were identified based on the comparison with reference specimens from the Forest Research Institute Malaysia Herbarium. The equipment used for hydrodistillation extraction process is Soxhlet extraction apparatus and mini rotary evaporator. Prior to the analysis, all samples were ground using Retsch Ultra Centrifugal Mill ZM 200. To characterize the sample, Vion IMS QTof 1.0 was used to quantify chemical compounds, and Nicolet iS50 FTIR Spectrometer was used to identify functional groups.

2.2 Sample preparation

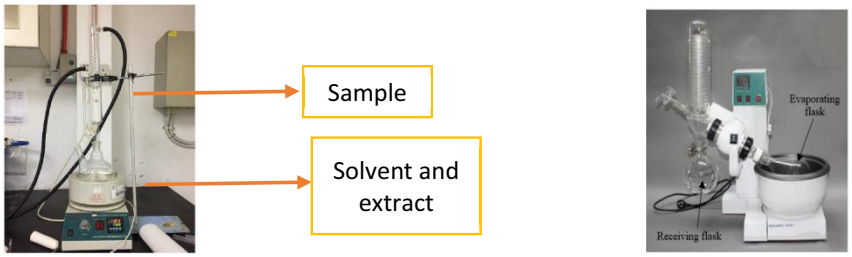
The fruits were dried in the oven at 60°C for one day and ground into powder using the Retsch Ultra Centrifugal Mill ZM 200 to 0.5 cm to 1 cm. Then, 40g of ground sample was weighed and put inside a beaker for further soaking with 1L of distilled water for 7 days, at room temperature. The purpose of this process is to break the parenchyma cells so that it will facilitate the oil glands rupture and consequently easier to extract the hydrosol.

2.3 Hydrodistillation extraction process

For the extraction process, Soxhlet extraction apparatus and mini rotary evaporator were set up as in Figure 1(a) and (b), respectively. The ground fruits were placed inside the cellulose thimble before it was inserted into the extraction chamber. The experiment took place for 6 hours (24 cycles) at 100°C. Then, it was left for an hour to cool before the extracted sample was collected. Then, the process was continued to separate the extracted sample using a mini rotary evaporator at 80°C. Hydrosol sample were collected in the receiving flask while concentrated agarwood extracts remained in the evaporating flask. The parameters was selected based on literature.

2.4 Liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS)

The hydrosol sample from the mini rotary evaporator was analyzed using liquid chromatography quadrupole time of flight mass spectrometry of Vion IMS QTof 1.0 series liquid chromatography. Negative electrospray ionization (ESI-) and Positive electrospray ionization were set up to identify the compounds in the hydrosol sample. The analytical run was set at 20 minutes, and the flow profile of the mobile phase is shown in Table 1. Other parameters of the system are summarized in Table 2. The identification of the compounds present in hydrosol samples was performed by comparing with MS/MS spectra from literature and records from the METLIN database. The tentative identification of some derivatives was based on the fragmentation patterns of known compounds.



(a) (b)

Figure 1. (a) Soxhlet extraction apparatus set up for extraction process (b) Rotary evaporation set up for separation process

Table 1. Isocratic and gradient flow profiles of the mobile phase.

Time (min)	Flow Rate (mL/min)	Composition A [Water +0.1%Formic Acid (%)	Composition B [Acetonitrile (%)]	Curve
0	0.5	99	1	Initial
0.5	0.5	99	1	6
16	0.5	65	35	6
18	0.5	0	100	1
20	0.5	99	1	1

Table 2. Parameters of the LC-QTOF-MS/MS system.

	Acquisition Parameter	
Source type	Electrospray ionization	
Ion polarity	Negative	Positive
Scan	100-1000 m/z	50-1000 m/z
Collision energy	4.00-45.00 eV	4.00-40.00 eV
Set capillary	2.5kV	
Source temperature	120 °C	
Desolvation temperature	550 °C	
Desolvation gas	800 L/h	
Cone gas	50 L/hour	

2.5 Functional Group Analysis

All spectra were obtained using a Fourier Transform Infrared Spectroscopy (FTIR) with attenuated total reflectance on crystal and knob of the Nicole i50. Sample analysis was carried out in the spectral range 4000 to 400 cm^{-1}, and the signal was subjected to 32 scans at a resolution of 4.0 cm^{-1}.

3. Result and discussion

3.1 Chemical profiling of hydrosol from A. malaccensis fruit via LC-QTOF-MS/MS

Based on table A1 and table A2, negative ionization mode shows more detection of chemical compounds in Agarwood hydrosol compared to positive mode. These findings were in agreement with Steckel & Schlosser (2019), as negative ion mode (ES-) were importantly used for the characterisation of

flavonoids (polyphenol), oligosaccharides, carboxylic acids, sulphonamides, oligonucleotides, and rarely peptides [7]. There are 128 active chemical compounds founds in negative ionization mode. In comparison, only one chemical compound can be found when the mode was switched to positive ionization mode.

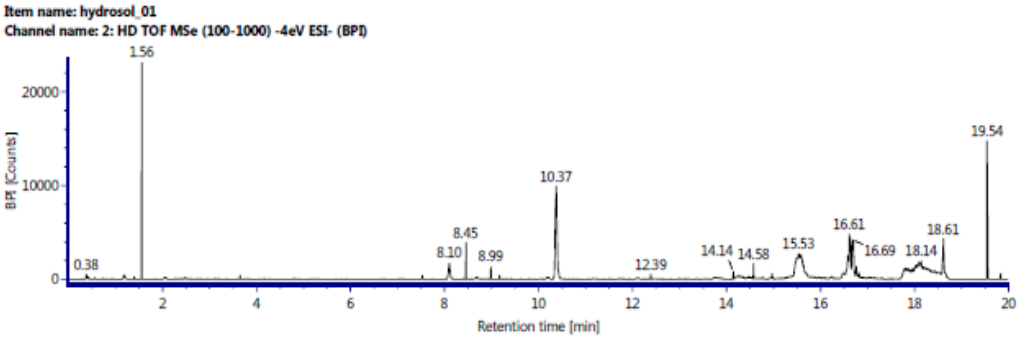
Referring to the study by Kruve et al. (2014), ions in negative ionization mode were generated via deprotonation, adduct formation with anions or via simultaneous deprotonation and adduct formation with cations [8]. Based on figure 2, the results obtained revealed some important biomarkers such as Decaffeoylacteoside (m/z=461.1667; Rt=11.08 min), Moracin C (m/z=355.1180; Rt= 12.29 min), Eugenol (m/z= 209.0817 ; Rt= 13.81 min), Dendrocandin B (m/z= 527.1940; Rt=15.53 min) and Xanthohumol (m/z= 353.1384 ; Rt= 16.61 min). The results from the mass-to-charge ratio revealed that Moracin C ($C_{19}H_{18}O_4$) was one of the most abundant phenolics compounds with inherent cancer capacity and potent antibacterial activity. Khyade & Lonkar (2013) investigated the inhibitory effect of Moracin C on cell mouse skin tumerigenesis model. It was found that Moracin can be used in cancer treatment when double dosage of 12-O-tetradecanoylphorbol 13-acetate (TPA) has been applied [9]. Hence, the utilisation of Moracin C may open a new avenue in the treatment of tumerigenesis.

Moreover, the second most abundant phenolics constituent was Decaffeoylacteoside ($C_20H_{30}O_{12}$) with antioxidant capacity. Decaffeoylacteoside has been utilised in traditional Chinese medicine to reduce heat from blood and disintegrate agglomerate [10]. Eugenol ($C_{10}H_{12}O_2$) known as clove oil was widely used as a flavouring for foods, and as a herbal oil used topically to treat toothache. Eugenol is one of the phenolic compounds found in essential oils or hydrosol [11].

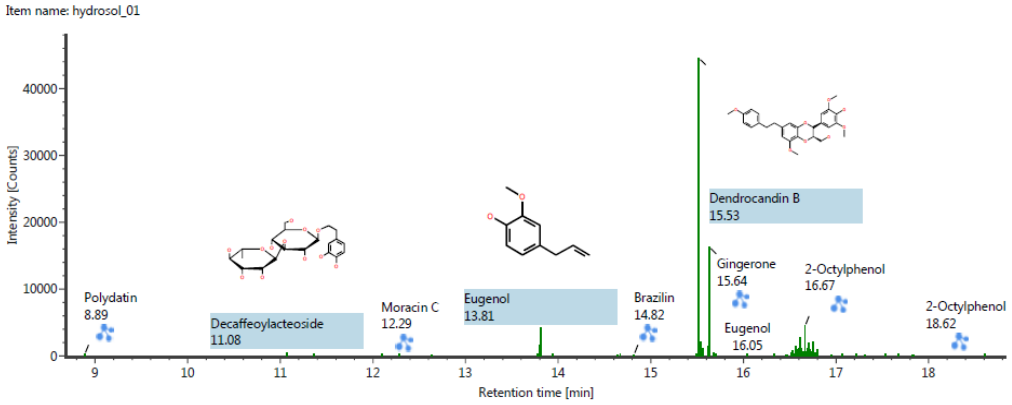
Another phenolic compound that was found in this study is Dendrocandin B ($C_{27}H_{30}O_8$). Mittraphab et al., (2016) reported that Dendrocandin B acts as a cell-killing agent against three human cancer cell lines, including MDA-231(Breast cancer cell line), HepG2 and HT-29 (Colorectal tumour cells) [12]. Hence, the cumulative of bioactivities of chemical profile found in these studies are primarily responsible for the numerous therapeutic functions. The result described from this investigation can be used for further studies into other nutraceutical or food applications of agarwood hydrosol from *Aquilaria malaccensis* fruits.

3.2 Functional Group Analysis

Result obtained via Fourier transform infrared spectroscopy illustrated that the hydrosol sample from the experiment has a similar peak with commercial agarwood hydrosol. The result also showed both sample present similar active functional groups; alcohol/phenol, alkyne, and amide I. Commonly, alcohol/phenol band group was characterised in the frequency range of 3600 cm^{-1} to 3200cm^{-1} [13]. From the broad spectrum in figure 3, O-H bond which indicates the presence of alcohol/phenol band group was found in the concentrated experimental agarwood hydrosol and industrial-grade agarwood hydrosol, at a wavenumber of 3270.59 cm^{-1} and 3272.60 cm^{-1}, respectively. Since both samples has strong H-bond, therefore the frequency becomes lower. The presence of the alcohol/phenols functional groups are significant to prove the existence of a phenolic compound inside the experimental hydrosol sample. This finding is in agreement with Khalil et al. (2013), where the phenolic compound in *Aquilaria malaccensis* leaves with the frequency of 3388 cm^{-1} was detected. The C≡C bond of alkyne group frequency (2260 cm^{-1} to 2100 cm^{-1}) was present in both commercial hydrosol sample and experimental hydrosol extract with the frequency of 2136.97 cm^{-1} and 2137.04 cm^{-1}, respectively [14]. Amides bands were also found with N-H bending at frequency of 1650 cm^{-1} -1560 cm^{-1}, identified in both leaves and hydrosol extracts with the frequency of 1635.08cm^{-1} and 1635.05cm^{-1}, respectively. The presence of amide indicated the existence of protein inside the agarwood hydrosol sample.

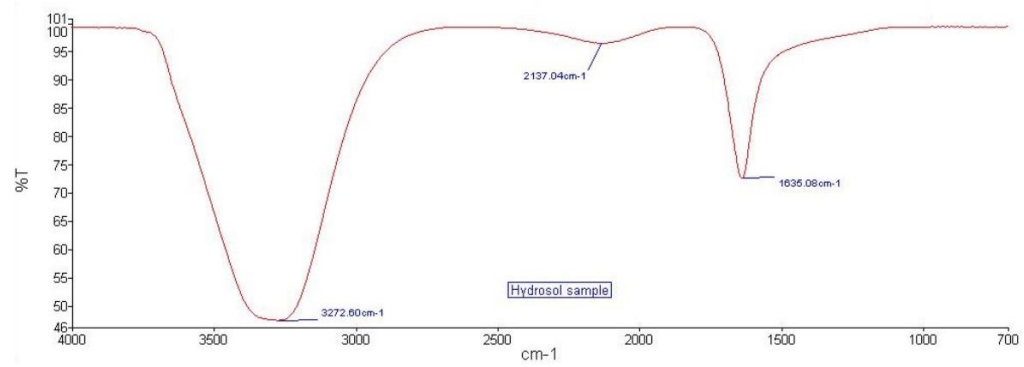


(a)

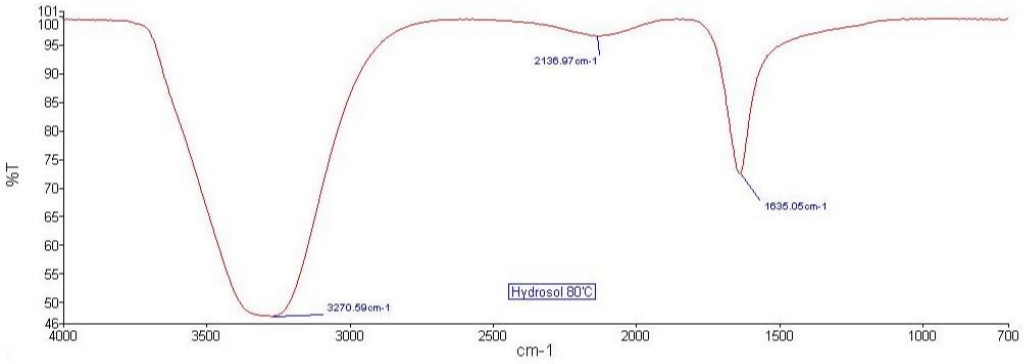


(b)

Figure 2. LC-QTOF chromatogram (negative ionization mode) of hydrosol from agarwood fruit extracts (a) BPI plot (b) Confirmed phenolic compound.



(a)



(b)

Figure 3. Overlaid of FTIR spectra (a) commercialise agarwood hydrosol (b) experiment agarwood hydrosol (temperature 80°C).

4. Conclusion

The data obtained from liquid chromatography quadrupole time of flight mass spectrometry found 128 untargeted compounds (known compound) in negative ionization mode and only one chemical profile detected after switching to positive ionization mode. From this data, we conclude that agarwood hydrosols from *A. malaccensis* fruit possesses many bioactive compounds useful for health. Agarwood hydrosol and fruits could be new resources for bioactive compounds and can stand as a potential halal and safe ingredients for the development of food, neutraceutical and pharmaceutical as well as cosmeceutical products. This study can help researchers in designing fractionation and insulation for effective drug discovery assays for new therapeutic application from agarwood hydrosol.

Appendices

Table A1. Chemical compounds detected in Agarwood hydrosol at temperature 80°C via negative mode LC-QTOF MS.

No	Compound	Formula	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed Retention Time (min)	Response	Adducts	Observed CCS (Å^2)	Total Fragments Found
1	Polydatin	$C_{20}H_{22}O_8$	390.1333	435.1315	1.8	8.89	188	+HCOO	199.06	0
2	Decaffeoylacteoside	$C_{20}H_{30}O_{12}$	462.1739	461.1667	0.2	11.08	472	-H	198.79	0
3	2-Hydorxy-5-methyl-hypnone	$C_9H_{10}O_2$	150.0684	195.0666	0.3	11.36	305	+HCOO	139.81	0
4	Yakuchinone A	$C_{20}H_{24}O_3$	312.1735	357.1717	1.0	12.10	299	+HCOO	195.49	0
5	Moracin C	$C_{19}H_{18}O_4$	310.1198	355.1180	-0.7	12.29	177	+HCOO	192.31	0
6	2,7-Dihydroxy-3,5-dimethoxy-9,10-dihydrophenanthrene	$C_{16}H_{16}O_4$	272.1043	271.0970	-0.6	12.63	151	-H	166.47	0
7	Gingerone	$C_{11}H_{14}O_3$	194.0941	193.0868	-0.2	13.78	258	-H	144.76	0
8	Dihydroeugenol	$C_{10}H_{14}O_2$	166.0993	165.0921	0.0	13.80	1585	-H	145.09	0
9	Eugenol	$C_{10}H_{12}O_2$	164.0835	209.0817	-0.2	13.81	4130	+HCOO	146.20	0
10	Obtustyrene	$C_{16}H_{16}O_2$	240.1146	285.1128	-0.4	13.93	172	+HCOO	172.42	0
11	Moscatilin	$C_{17}H_{20}O_5$	304.1302	349.1284	-0.9	14.63	115	+HCOO	181.48	0
12	Eugenol	$C_{10}H_{12}O_2$	164.0836	209.0818	-0.2	14.67	210	+HCOO	146.85	0

No	Compound	Formula	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed Retention Time (min)	Response	Adducts	Observed CCS (Å^2)	Total Fragments Found
13	Brazilin	$C_{16}H_{14}O_5$	286.0843	285.0770	0.2	14.82	164	-H	171.39	1
14	Blestriarene C	$C_{30}H_{22}O_6$	478.1408	523.1390	-0.8	15.50	196	+HCOO	216.71	0
15	Dendrocandin B	$C_{27}H_{30}O_8$	482.1958	527.1940	1.7	15.53	44529	+HCOO	219.77	11
16	Dendrocandin B	$C_{27}H_{30}O_8$	482.1936	527.1918	-0.5	15.53	4140	+HCOO	221.15	11
17	Cistanoside H	$C_{22}H_{32}O_{13}$	504.1837	503.1764	-0.6	15.53	308	-H	212.43	10
18	2-Hydroxy-5-methyl-hypnone	$C_9H_{10}O_2$	150.0681	149.0608	0.0	15.53	358	-H	135.22	0
19	Blestrianol D	$C_{29}H_{24}O_5$	452.1632	497.1614	0.8	15.54	770	+HCOO	223.66	2
20	2-Ethyl-4,5-dimethyl-phenol	$C_{10}H_{14}O$	150.1046	149.0974	0.2	15.54	1981	-H	137.54	0
21	Eugenol	$C_{10}H_{12}O_2$	164.0837	163.0764	0.0	15.54	173	-H	139.24	1
22	2-Ethyl-4,5-dimethyl-phenol	$C_{10}H_{14}O$	150.1047	149.0974	0.2	15.57	1019	-H	163.02	0
23	Tran-Ferulaldehyde	$C_{10}H_{10}O_3$	178.0629	177.0556	-0.1	15.62	1383	-H	134.07	0
24	Gingerone	$C_{11}H_{14}O_3$	194.0942	193.0869	-0.1	15.64	16248	-H	143.49	1
25	Aspidinol	$C_{12}H_{16}O_4$	224.1048	223.0975	-0.1	15.68	386	-H	154.25	0
26	Stilbostemin B	$C_{15}H_{16}O_2$	228.1157	273.1139	0.6	15.70	232	+HCOO	174.22	0
27	Eugenol	$C_{10}H_{12}O_2$	164.0831	209.0813	-0.6	16.05	172	+HCOO	147.43	0
28	Moracin C	$C_{19}H_{18}O_4$	310.1201	355.1183	-0.5	16.33	183	+HCOO	192.36	0
29	Isomucronustyrene	$C_{17}H_{18}O_3$	270.1262	269.1189	0.6	16.45	104	-H	169.27	0
30	Dendrocandin C	$C_{16}H_{18}O_5$	290.1141	289.1068	-1.4	16.47	153	-H	179.29	0
31	2-Ethyl-4,5-dimethyl-phenol	$C_{10}H_{14}O$	150.1045	149.0973	0.1	16.52	526	-H	162.71	0
32	2-Ethyl-4,5-dimethyl-phenol	$C_{10}H_{14}O$	150.1045	149.0972	0.0	16.53	717	-H	137.33	0
33	Cishinokiresinol	$C_{17}H_{16}O_2$	252.1145	251.1072	-0.5	16.55	211	-H	162.78	0

No	Compound	Formula	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed Retention Time (min)	Response	Adducts	Observed CCS (Å^2)	Total Fragments Found
34	Flavanthrinin	$C_{15}H_{12}O_3$	240.0784	239.0711	-0.2	16.56	1363	-H	153.47	2
35	2-Octylphenol	$C_{14}H_{22}O$	206.1671	251.1653	0.0	16.57	106	+HCOO	165.18	2
36	Effusol	$C_{17}H_{16}O_2$	252.1142	251.1069	-0.8	16.57	161	-H, +HCOO	154.92	2
37	Moracin O	$C_{19}H_{18}O_5$	326.1145	325.1072	-1.0	16.57	499	-H	180.15	3
38	Eugenol	$C_{10}H_{12}O_2$	164.0830	209.0812	-0.7	16.58	241	+HCOO	143.34	0
39	3-Hydroxy-1-(4- hydroxy-3- methoxyphenyl)-2-propanone	$C_{10}H_{12}O_4$	196.0728	195.0655	-0.7	16.58	88	-H	137.67	1
40	Oxyphyllacinol	$C_{20}H_{26}O_3$	314.1864	359.1846	-1.8	16.58	345	+HCOO	191.88	14
41	Aspidinol	$C_{12}H_{16}O_4$	224.1033	223.0960	-1.6	16.58	139	-H	149.26	3
42	4'-Methylpinosylvin	$C_{15}H_{14}O_2$	226.0986	225.0914	-0.7	16.58	211	-H, +HCOO	152.83	0
43	Shogaol	$C_{17}H_{24}O_3$	276.1715	321.1697	-1.0	16.58	252	+HCOO	181.50	1
44	Agrimol E	$C_{33}H_{38}O_{12}$	626.2360	671.2342	-0.4	16.58	878	+HCOO	246.01	0
45	Feralolide	$C_{18}H_{16}O_7$	344.0899	343.0826	0.3	16.58	277	-H	177.72	2
46	Yakuchinone A	$C_{20}H_{24}O_3$	312.1745	311.1672	2.0	16.58	232	-H	175.74	1
47	1-O-Methyl-3,5-O-dicaffeoylquinic acid methyl ester	$C_{27}H_{28}O_{12}$	544.1576	543.1503	-0.5	16.59	313	-H	219.20	4
48	Obtustyrene	$C_{16}H_{16}O_2$	240.1151	285.1133	0.1	16.59	241	+HCOO	170.52	0
49	4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one	$C_{12}H_{14}O_4$	222.0894	221.0821	0.2	16.59	125	-H	147.45	1
50	Cyclocurcumin	$C_{21}H_{20}O_6$	368.1252	367.1179	-0.8	16.59	433	-H	190.15	9

No	Compound	Formula	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed Retention Time (min)	Response	Adducts	Observed CCS (Å^2)	Total Fragments Found
51	Erianin	$C_{18}H_{22}O_5$	318.1457	317.1384	-1.1	16.59	640	-H	181.57	3
52	Isoscoparone	$C_{17}H_{18}O_4$	286.1194	331.1176	-1.1	16.59	254	+HCOO, -H	187.29	6
53	(±)-Vestitol	$C_{16}H_{16}O_4$	272.1051	271.0978	0.2	16.59	135	-H	161.81	3
54	Agrimol D	$C_{35}H_{42}O_{12}$	654.2670	699.2652	-0.7	16.59	574	+HCOO	253.10	1
55	Isoarundinin II	$C_{22}H_{22}O_4$	350.1499	349.1426	-2.0	16.60	687	-H	193.86	5
56	tran-Ferulaldehyde	$C_{10}H_{10}O_3$	178.0632	177.0559	0.2	16.60	253	-H	135.58	0
57	Obovatol	$C_{18}H_{18}O_3$	282.1242	327.1224	-1.4	16.60	502	+HCOO, -H	180.66	2
58	Neosappanone A	$C_{33}H_{28}O_{11}$	600.1625	599.1552	-0.7	16.60	615	-H	234.15	3
59	6-Gingerol	$C_{17}H_{26}O_4$	294.1830	293.1757	-0.1	16.60	472	-H	304.72	3
60	Dendrobin A	$C_{16}H_{18}O_4$	274.1196	273.1123	-0.9	16.60	109	-H	165.45	4
61	2,7-Dihydroxy-1-(p-hydroxybenzoyl)-4-methoxy-9,10-dihydrophenanthrene	$C_{22}H_{18}O_5$	362.1159	361.1086	0.5	16.60	420	-H	191.68	6
62	Moscatilin	$C_{17}H_{20}O_5$	304.1322	303.1249	1.1	16.60	289	-H	175.16	4
63	7-(4-Hydroxy-3-methoxyphenyl)-I-(4-hydroxyphenyl)-4E,6E-heptadien-3-one	$C_{20}H_{20}O_4$	324.1349	323.1276	-1.2	16.60	605	-H	183.65	3
64	Euparin	$C_{13}H_{12}O_3$	216.0777	215.0704	-1.0	16.60	427	-H, +HCOO	147.65	0
65	Dihydroresveratrol	$C_{14}H_{14}O_3$	230.0957	229.0884	1.4	16.61	174	-H	153.01	1
66	Moracin C	$C_{19}H_{18}O_4$	310.1213	355.1195	0.8	16.61	1106	+HCOO	188.47	5
67	Dendrocandin D	$C_{17}H_{20}O_5$	304.1299	303.1227	-1.1	16.61	129	-H	191.73	8

No	Compound	Formula	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed Retention Time (min)	Response	Adducts	Observed CCS (Å^2)	Total Fragments Found
68	Agrimol C	$C_{36}H_{44}O_{12}$	668.2842	713.2825	1.0	16.61	662	+HCOO	256.46	1
69	4,7-Dihydroxy-1-(p-hydroxybenzyl)-2- methoxy-9,10-dihydrophenanthrene	$C_{22}H_{20}O_4$	348.1361	347.1288	0.0	16.61	854	-H	187.27	9
70	Xanthohumol	$C_{21}H_{22}O_5$	354.1457	353.1384	-1.0	16.61	1324	-H	191.03	17
71	Bletilol C	$C_{27}H_{26}O_7$	462.1686	461.1613	0.7	16.62	1013	-H	210.33	18
72	4,7-Dihydroxy-1-(p-hydroxybenzyl)-2- methoxy-9,10-dihydrophenanthrene	$C_{22}H_{20}O_4$	348.1361	347.1288	-0.1	16.62	1494	-H, +HCOO	193.27	7
73	2-Hydroxyphenylpropanol	$C_9H_{12}O_2$	152.0837	151.0764	-0.1	16.62	222	-H	139.22	0
74	2,4-Dihydroxyaceto-phenone	$C_8H_8O_3$	152.0482	151.0409	0.9	16.62	87	-H	158.41	0
75	6-Gingerdione	$C_{17}H_{24}O_4$	292.1682	291.1609	0.7	16.62	1144	-H	168.20	8
76	2,6-Di-tert-butyl-4-hydroxy toluene	$C_{15}H_{24}O$	220.1830	265.1812	0.3	16.62	2754	+HCOO	165.19	1
77	Polygoacetophenoside	$C_{14}H_{18}O_{10}$	346.0884	391.0866	-1.6	16.62	1867	+HCOO	186.75	3
78	Isoarundinin II	$C_{22}H_{22}O_4$	350.1510	395.1492	-0.8	16.63	1092	+HCOO, -H	199.59	6
79	Moracin H	$C_{20}H_{18}O_5$	338.1153	383.1135	-0.1	16.63	419	+HCOO, -H	191.22	22
80	1,7-Bis(4-hydroxyphenyl)-hepta- 4E,6E-dien-3-one	$C_{19}H_{18}O_3$	294.1239	293.1166	-1.7	16.63	135	-H	175.00	0

No	Compound	Formula	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed Retention Time (min)	Response	Adducts	Observed CCS (Å^2)	Total Fragments Found
81	Gigantol	$C_{15}H_{16}O_4$	260.1041	259.0968	-0.8	16.64	230	-H	157.62	1
82	Pseudoaspidin	$C_{25}H_{32}O_8$	460.2079	459.2006	-1.8	16.64	599	-H	216.24	10
83	6-Gingerol	$C_{17}H_{26}O_4$	294.1830	293.1757	-0.1	16.65	367	-H	175.77	5
84	Dendrocandin E	$C_{15}H_{16}O_5$	276.0999	275.0926	0.1	16.65	386	-H	209.73	1
85	2-Octylphenol	$C_{14}H_{22}O$	206.1672	205.1599	0.1	16.67	4473	-H, +HCOO	155.29	0
86	Dihydrocurcumin	$C_{21}H_{22}O_6$	370.1411	369.1338	-0.5	16.67	560	-H	192.01	15
87	Yakuchinone B	$C_{20}H_{22}O_3$	310.1566	355.1548	-0.3	16.67	2733	+HCOO	187.44	1
88	2-Ethyl-4,5-dimethyl-phenol	$C_{10}H_{14}O$	150.1043	149.0970	-0.2	16.68	455	-H	147.32	0
89	Dendrobin A	$C_{16}H_{18}O_4$	274.1187	273.1114	-1.8	16.68	190	-H	168.70	1
90	(3R)-Duartin	$C_{18}H_{20}O_6$	332.1246	331.1173	-1.4	16.68	348	-H	178.51	1
91	Shogaol	$C_{17}H_{24}O_3$	276.1731	321.1713	0.5	16.69	576	+HCOO, -H	185.95	3
92	Dihydroeugenol	$C_{10}H_{14}O_2$	166.0989	165.0916	-0.5	16.69	83	-H	138.82	0
93	Obovatol	$C_{18}H_{18}O_3$	282.1255	327.1237	-0.1	16.70	461	+HCOO, -H	180.61	1
94	Oxyphyllacinol	$C_{20}H_{26}O_3$	314.1876	313.1803	-0.6	16.70	414	-H	199.59	7
95	2-Octylphenol	$C_{14}H_{22}O$	206.1672	205.1599	0.1	16.70	96	-H	210.54	3
96	(3R,4R)-3,4-trans-7,2',3'-Ttrihydroxy-4'-methoxy-4-[(3R)-2',7-dihydroxy-4'-methoxy-isoflavan-5'-yl]-isoflavan	$C_{32}H_{30}O_9$	558.1877	603.1859	-1.3	16.70	993	+HCOO	237.57	4
97	Octahydrocurcumin	$C_{21}H_{28}O_6$	376.1879	375.1807	-0.7	16.70	239	-H	313.90	2

No	Compound	Formula	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed Retention Time (min)	Response	Adducts	Observed CCS (Å^2)	Total Fragments Found
98	Dendrocandin C	C16H18O5	290.1138	289.1065	-1.6	16.70	112	-H	193.63	1
99	(3R,4R)-3,4-trans-7,2’,3’-Ttrihydroxy-4’-methoxy-4-[(3R)-2’,7-dihydroxy-4’-methoxy-isoflavan-5’-yl]-isoflavan	C32H30O8	542.1930	587.1912	-1.1	16.70	951	+HCOO	243.24	3
100	Blestriarene B	C30H24O6	480.1557	525.1539	-1.6	16.71	1053	+HCOO	221.97	8
101	Mulberrofuran N	C25H28O4	392.1972	437.1954	-1.6	16.71	1816	+HCOO	209.88	11
102	5-O-Methylshanciguol	C29H28O5	456.1920	501.1902	-1.7	16.71	560	+HCOO	217.58	3
103	3’-O-Methylbrazilin	C17H16O5	300.1015	299.0942	1.7	16.71	220	-H	164.66	5
104	Blestrianol D	C29H24O5	452.1641	497.1623	1.7	16.71	820	+HCOO	222.35	1
105	2,7-Dihydroxy-1-(p-hydroxybenzoyl)-4-methoxy-9,10-dihydrophenanthrene	C22H18O5	362.1136	361.1063	-1.9	16.72	844	-H	185.95	5
106	Decaffeoylacteoside	C20H30O12	462.1724	507.1706	-1.3	16.72	372	+HCOO	215.63	15
107	Kuwanon P	C34H30O9	582.1879	627.1861	-1.1	16.73	698	+HCOO	244.10	4
108	Mulberrofuran O	C39H34O9	646.2192	691.2174	-1.1	16.73	383	+HCOO	260.97	0
109	2-Octylphenol	C14H22O	206.1672	205.1600	0.2	16.74	627	-H	180.40	0
110	1-Galloyl-β-D-glucose	C13H16O10	332.0727	377.0709	-1.7	16.74	139	+HCOO	322.64	0
111	Dihydrocurcumin	C21H22O6	370.1426	369.1354	1.0	16.76	783	-H	188.93	3
112	1-Galloyl-β-D-glucose	C13H16O10	332.0726	377.0708	-1.8	16.76	1971	+HCOO	184.31	0
113	Dihydroreveratrol	C14H14O3	230.0941	275.0923	-0.2	16.76	257	+HCOO	224.54	0
114	Dihydrooxyresveratrol	C14H14O4	246.0878	291.0860	-1.4	16.76	103	+HCOO	219.72	0
115	6-Gingerol	C17H26O4	294.1840	293.1767	0.9	16.77	134	-H	179.67	2

No	Compound	Formula	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed Retention Time (min)	Response	Adducts	Observed CCS (Å^2)	Total Fragments Found
116	4,7-Dihydroxy-1-(p-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene	C22H20O4	348.1374	393.1356	1.3	16.79	392	+HCOO	194.88	1
117	Xanthohumol	C21H22O5	354.1465	353.1392	-0.3	16.80	838	-H	189.00	1
118	4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one	C12H14O4	222.0891	221.0818	-0.1	16.95	110	-H	149.91	0
119	3,7-Dihydroxy-2,4-dimethoxyphenanthrene-3-O-glucoside	C22H24O9	432.1418	431.1345	-0.3	17.07	267	-H	206.25	0
120	7-(4-Hydroxy-3-methoxyphenyl)-I-(4-hydroxyphenyl)-4E,6E-heptadien-3-one	C20H20O4	324.1362	369.1344	0.0	17.22	366	+HCOO	201.51	0
121	Isoarundinin II	C22H22O4	350.1505	349.1433	-1.3	17.32	93	-H	205.73	0
122	Cyclocurcumin	C21H20O6	368.1261	367.1188	0.1	17.54	259	-H	196.17	0
123	Chrysotoxine	C18H22O5	318.1466	317.1393	-0.2	17.68	185	-H	183.91	0
124	2-Octylphenol	C14H22O	206.1672	205.1600	0.2	17.83	165	-H	158.76	0
125	2-Octylphenol	C14H22O	206.1668	205.1595	-0.3	18.62	287	-H	156.36	0
126	Parvifloroside B	C29H36O15	624.2036	623.1963	-1.8	18.62	125	-H	258.61	0
127	Cyclocurcumin	C21H20O6	368.1245	367.1172	-1.5	18.62	82	-H	199.66	1
128	Octahydrocurcumin	C21H28O6	376.1888	375.1816	0.3	18.64	87	-H	196.86	7

Table A2. Chemical compounds detected in Agarwood hydrosol at temperature 80°C via positive mode LC-QTOF MS.

No	Compound	Formula	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed Retention Time (min)	Response	Adducts	Observed CCS (Å2)	Total Fragments Found
1	Caffeate 1	C9H8O4	180.0428	181.0500	0.5	16.61	149	+H	135.63	0

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